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sequence_AA_2D12.5_variable domains.txt

```
>2D12.5VL_MOUSE
(1) QAVVTQESALTSPGETVTLICRSTGAVTTSNYANWVQEKPDHLFTGLIGNNNRPPGVPARFSGSLIGDKAALTIAGTQTED
    EAIYFCALWYSNHWVFGGGTRLTVLG

(2) CDR1 - RSSTGAVTTSNYAN
(3) CDR2 - GNNNRPP
(4) CDR3 - ALWYSNHWV

>2D12.5VH_MOUSE
(5) QVKLQESGPGVLQPSQSLSTCTVSGFSLTDYGVHWVRQSPGKGLEWLGVIWSGGGTAYTAAAFISRLNIY
    KDNSKNQVFFEMNSLOANDTAMYYCARRGSPYNYFDVWGQGTTVTVSS

(6) CDR1 - DYGVH
(7) CDR2 - VIWSGGGTAYTAAAFIS
(8) CDR3 - RGSYPYNYFDV
```

FIG. 1

Translation of 2d12.5 VH variable genes

(9)2d12.5 VH native hybridoma	1	VKLQESGPGLVQPSQSLSITCTVSGFSLTDYGVHWV RQSPGKGLEWLGVI	50
(10)2d12.5 VH native cloned	1	50
(11)2d12.5 VH N87D_cloned	1	50
(12)2d12.5 VH N87D_G53C_cloned	1	50
(13)2d12.5 VH N87D_G54C_cloned	1	50
(14)2d12.5 VH N87D_G55C_cloned	1	50

2d12.5 VH native hybridoma	51	WSGGGTAYTAAFISRLNIYKDNSKNQVFFEMNSLQANDTAMYCARRGSY	100
2d12.5 VH native cloned	51	100
2d12.5 VH N87D_cloned	51D.....	100
2d12.5 VH N87D_G53C_cloned	51	...C.....D.....	100
2d12.5 VH N87D_G54C_cloned	51	...C.....D.....	100
2d12.5 VH N87D_G55C_cloned	51	...C.....D.....	100

2d12.5 VH native hybridoma	101	PNYFDVWGQGTTVTVSS	118
2d12.5 VH native cloned	101A	118
2d12.5 VH N87D_cloned	101A	118
2d12.5 VH N87D_G53C_cloned	101A	118
2d12.5 VH N87D_G54C_cloned	101A	118
2d12.5 VH N87D_G55C_cloned	101A	118

FIG. 2

2D12.5 VH variable genes

(15) 2d12.5 VH native hybridoma	1	GTGAAGCTGCAGGAGTCAGGACCTGGCCCTAGTCAGCCCTCAGAGCCT	50
(16) 2d12.5 VH native cloned	1T.....	50
(17) 2d12.5 VH N87D cloned	1T.....	50
(18) 2d12.5 VH N87D_G53C cloned	1T.....	50
(19) 2d12.5 VH N87D_G54C cloned	1T.G.....	50
(20) 2d12.5 VH N87D_G55C cloned	1T.....	50

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2d12.5 VH native hybridoma	51	GTCCATCACCTGCACGGTCTCTGGTTTCTCATTAAGTACTATGGTGCTAC	100
2d12.5 VH native cloned	51	100
2d12.5 VH N87D cloned	51	100
2d12.5 VH N87D_G53C cloned	51	100
2d12.5 VH N87D_G54C cloned	51	100
2d12.5 VH N87D_G55C cloned	51	100

2d12.5 VH native hybridoma	101	ACTGGGTTCCGCAGTCTCCAGGAAGGGTCTGGAAATGGCTGGGAGTGATA	150
2d12.5 VH native cloned	101	150
2d12.5 VH N87D cloned	101	150
2d12.5 VH N87D_G53C cloned	101	150
2d12.5 VH N87D_G54C cloned	101	150
2d12.5 VH N87D_G55C cloned	101	150

FIG. 3A

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FIG. 3A (CONT.)

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	310	320	330	340	350
				
2d12.5 VH native hybridoma	301 CCTTACA	ACTTCTG	ATGCTG	GGCCAA	GGGACCA
2d12.5 VH native cloned	301				AGTCACCGTCTC
2d12.5 VH N87D cloned	301				G.....
2d12.5 VH N87D_G53C cloned	301				G.....
2d12.5 VH N87D_G54C cloned	301				G.....
2d12.5 VH N87D_G55C cloned	301				G.....

FIG. 3A (CONT.)

2D12.5 VH variable genes

2d12.5 VH native hybridoma	351	CTCA	354
2d12.5 VH native cloned	351	.G..	354
2d12.5 VH N87D cloned	351	.G..	354
2d12.5 VH N87D_G53C cloned	351	.G..	354
2d12.5 VH N87D_G54C cloned	351	.G..	354
2d12.5 VH N87D_G55C cloned	351	.G..	354

FIG. 3B

Translation of 2D12.5 VL genes

		10	20	30	40	50	
						
(21)2d12.5 VL native hybridoma	1	AVVTQESALTTSPGETVTLTCRSSSTGAVTTSNYANWVQEKPDHLFTGLIG	50				
(22)2d12.5 VL native cloned	1	50				
(23)2d12.5 VL N53C_cloned	1	50				
		60	70	80	90	100	
						
2d12.5 VL native hybridoma	51	GNNRPPGVPARFSGSLIGDKAALTIAGTQTEDEAIYFCALWYSNHWVFG	100				6/41
2d12.5 VL native cloned	51	100				
2d12.5 VL N53C_cloned	51	100				
						
2d12.5 VL native hybridoma	101	GGTRLTVLG	109				
2d12.5 VL native cloned	101	...K....S	109				
2d12.5 VL N53C_cloned	101	...K....S	109				

FIG. 4

FIG. 5

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	210	220	230	240	250
2d12.5 VL native hybridoma				
	201	GATTGGAGACAAGGCTGCCCTCACCATCGCAGGACACAGACTGAGGATG			250
2d12.5 VL native cloned	201			250
2d12.5 VL N53C_cloned	201			250
	260	270	280	290	300
2d12.5 VL native hybridoma				
	251	AGGCAATATATTCTGTGCTCTATGGTACAGCAACCATTGGGTGTTTCGGT			300
2d12.5 VL native cloned	251			300
2d12.5 VL N53C_cloned	251			300
	310	320			
2d12.5 VL native hybridoma				
	301	GGAGGAACCAGACTGACTGTCTCCTAGGCC			327
2d12.5 VL native cloned	301	..G.....A.....			327
2d12.5 VL N53C_cloned	301	..G.....A.....			327

FIG. 5 (CONT.)

Translation of Mouse 2D12.5 VL - Human TetTox CL kappa (light chain gene)

		10	20	30	40	50																																							
																																												
(27)	2dVL-TTCL native_cloned	1	RS	AV	TQ	ES	ALT	T	S	P	G	E	T	V	T	L	T	C	R	S	S	T	G	A	V	T	S	N	Y	A	N	W	V	Q	E	K	P	D	H	L	F	T	G	L	50
(28)	2dVL-TTCL N53C_cloned	1																																									50	
(29)	2d12.5 VL native hybridoma	1	--																																								50		
(30)	TTCL template for gene assembly	1	----- ----- ----- ----- ----- ----- -----																																										

		60	70	80	90	100																																											
																																																
	2dVL-TTCL native_cloned	51	I	G	G	N	N	R	P	P	G	V	P	A	R	F	S	G	S	L	I	G	D	K	A	A	L	T	I	A	G	T	E	D	E	A	I	F	C	A	L	W	Y	S	N	H	W	V	100
	2dVL-TTCL N53C_cloned	51																																												100		
	2d12.5 VL native hybridoma	49																																												98		
	TTCL template for gene assembly	1	----- ----- ----- ----- ----- ----- -----																																														

		110	120	130	140	150																																														
																																																			
	2dVL-TTCL native_cloned	101	F	G	G	T	K	L	T	V	L	S	R	T	V	A	A	P	S	V	F	I	F	P	P	S	D	E	Q	L	K	S	G	T	A	S	V	V	C	L	L	N	N	F	Y	P	R	E	A	K	V	150
	2dVL-TTCL N53C_cloned	101																																														150			
	2d12.5 VL native hybridoma	99																																														100			
	TTCL template for gene assembly	1	----- ----- ----- ----- ----- ----- -----																																														80			

FIG. 6

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2dVL-TTCL native_cloned      210      220
      ....|.....|.....|.....|.....
201 THQGLSLPVTKSFNRGEC*F*221
2dVL-TTCL N53C_cloned      201      *221
2d12.5 VL native hybridoma
TTCL template for gene assembl 90 ..... 107

```

FIG. 6 (CONT.)

FIG. 7A

Mouse 2D12.5 VL - Human TetTox CL kappa (light chain gene)

	10	20	30	40	50
				
(31)2dVL-TTCL native_cloned	1				50
(32)2dVL-TTCL N53C_cloned	1				50
(33)2d12.5 VL native hybridoma	1				44
(34)TTCL template for gene assem	1				1
	60	70	80	90	100
				
2dVL-TTCL native_cloned	51				100
2dVL-TTCL N53C_cloned	51				100
2d12.5 VL native hybridoma	45				94
TTCL template for gene assem	1				1
	110	120	130	140	150
				
2dVL-TTCL native_cloned	101				150
2dVL-TTCL N53C_cloned	101				150
2d12.5 VL native hybridoma	95				144
TTCL template for gene assem	1				1
	160	170	180	190	200
				
2dVL-TTCL native_cloned	151				200
2dVL-TTCL N53C_cloned	151				200
2d12.5 VL native hybridoma	145				194
TTCL template for gene assem	1				1

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210      220      230      240      250
...|...|...|...|...|...|...|...|...|...|
201 CTCCCTGATTGGAGACAAGGCTGCCCTCACCATCGCAGGGACACAGACTG 250
201      250
...|...|...|...|...|...|...|...|...|...|
195      244
...|...|...|...|...|...|...|...|...|...|
TTCL template for gene assem 1 ----- 1

260      270      280      290      300
...|...|...|...|...|...|...|...|...|...|
251 AGGATGAGGCAATATATTCTGTGCTCTATGGTACAGCAACCATTGGGTG 300
251      300
...|...|...|...|...|...|...|...|...|...|
245      294
...|...|...|...|...|...|...|...|...|...|
TTCL template for gene assem 1 ----- 1

310      320      330      340      350
...|...|...|...|...|...|...|...|...|...|
301 TTCGGTGGGGGAACCAAACTGACTGTCTCTAAGCCGAACTGTGGCTGCACC 350
301      350
...|...|...|...|...|...|...|...|...|...|
295      327
...|...|...|...|...|...|...|...|...|...|
TTCL template for gene assem 1 ----- 17

360      370      380      390      400
...|...|...|...|...|...|...|...|...|...|
351 ATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAAC TG 400
351      400
...|...|...|...|...|...|...|...|...|...|
TTCL template for gene assem 18 ----- 67
```

FIG. 7A (CONT.)

	410	420	430	440	450
				
401	CCTCTGTTGTGTCCTGCTGAATAACTTCTATCCAGAGGCCAAAGTA	450			
401				
68	117			

TTCL template for gene assem

FIG. 7A (CONT.)

Mouse 2D12.5 VL - Human TetTox CL kappa (light chain gene)

	460	470	480	490	500
				
2dVL-TTCL native_cloned	451	CAGTGAAGTGGATAACGCCCTCCAATCGGGTAACCTCCAGGAGAGTGT			500
2dVL-TTCL N53C_cloned	451			500
2d12.5 VL native hybridoma				
TTCL template for gene assem	118			167
		510	520	530	540
				
2dVL-TTCL native_cloned	501	CACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCCTGA			550
2dVL-TTCL N53C_cloned	501			550
2d12.5 VL native hybridoma				
TTCL template for gene assem	168			217
		560	570	580	590
				
2dVL-TTCL native_cloned	551	CGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCCTGCCGAAGTC			600
2dVL-TTCL N53C_cloned	551			600
2d12.5 VL native hybridoma				
TTCL template for gene assem	218			267

FIG. 7B

	610	620	630	640	650	
					
2dVL-TTCL native_cloned	601	ACCCATCAGGGCCTGAGCTTGCCCGTCACAAAGAGCTTCAACAGGGGAGA	650			
2dVL-TTCL N53C_cloned	601T.....	650			
2d12.5 VL native hybridoma						
TTCL template for gene assem	268	317			
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	660					
					
2dVL-TTCL native_cloned	651	GTGTTAATTCTAGA	664			
2dVL-TTCL N53C_cloned	651	664			
2d12.5 VL native hybridoma						
TTCL template for gene assem	318	322			

FIG. 7B (CONT.)

Translation of Mouse 2D12.5 VH - Human TetTox CH1 (heavy chain Fab gene)

[illegible]

		210	220	
2dVH-TTCH_native cloned	201	YICNVNHHKPSNTKVDDKKAEPKSCDKSR			227
2dVH-TTCH_N87D_cloned	201			227
2dVH-TTCH_N87D_G53C_cloned	201			227
2dVH-TTCH_N87D_G54C_cloned	201			227
2dVH-TTCH_N87D_G55C_cloned	201			227
2dVH-TTCH expected sequence	201			227
2dI2.5 VH native hybridoma					

FIG. 8 (CONT.)

FIG. 9A

	1	20	40	60	80	100	120	140	160	180	200	220	240	260	280	300	320	340	360	380	400	420	440	460	480	500	520	540	560	580	600	620	640	660	680	700	720	740	760	780	800	820	840	860	880	900	920	940	960	980	1000	1020	1040	1060	1080	1100	1120	1140	1160	1180	1200	1220	1240	1260	1280	1300	1320	1340	1360	1380	1400	1420	1440	1460	1480	1500	1520	1540	1560	1580	1600	1620	1640	1660	1680	1700	1720	1740	1760	1780	1800	1820	1840	1860	1880	1900	1920	1940	1960	1980	2000	2020	2040	2060	2080	2100	2120	2140	2160	2180	2200	2220	2240	2260	2280	2300	2320	2340	2360	2380	2400	2420	2440	2460	2480	2500	2520	2540	2560	2580	2600	2620	2640	2660	2680	2700	2720	2740	2760	2780	2800	2820	2840	2860	2880	2900	2920	2940	2960	2980	3000	3020	3040	3060	3080	3100	3120	3140	3160	3180	3200	3220	3240	3260	3280	3300	3320	3340	3360	3380	3400	3420	3440	3460	3480	3500	3520	3540	3560	3580	3600	3620	3640	3660	3680	3700	3720	3740	3760	3780	3800	3820	3840	3860	3880	3900	3920	3940	3960	3980	4000	4020	4040	4060	4080	4100	4120	4140	4160	4180	4200	4220	4240	4260	4280	4300	4320	4340	4360	4380	4400	4420	4440	4460	4480	4500	4520	4540	4560	4580	4600	4620	4640	4660	4680	4700	4720	4740	4760	4780	4800	4820	4840	4860	4880	4900	4920	4940	4960	4980	5000	5020	5040	5060	5080	5100	5120	5140	5160	5180	5200	5220	5240	5260	5280	5300	5320	5340	5360	5380	5400	5420	5440	5460	5480	5500	5520	5540	5560	5580	5600	5620	5640	5660	5680	5700	5720	5740	5760	5780	5800	5820	5840	5860	5880	5900	5920	5940	5960	5980	6000	6020	6040	6060	6080	6100	6120	6140	6160	6180	6200	6220	6240	6260	6280	6300	6320	6340	6360	6380	6400	6420	6440	6460	6480	6500	6520	6540	6560	6580	6600	6620	6640	6660	6680	6700	6720	6740	6760	6780	6800	6820	6840	6860	6880	6900	6920	6940	6960	6980	7000	7020	7040	7060	7080	7100	7120	7140	7160	7180	7200	7220	7240	7260	7280	7300	7320	7340	7360	7380	7400	7420	7440	7460	7480	7500	7520	7540	7560	7580	7600	7620	7640	7660	7680	7700	7720	7740	7760	7780	7800	7820	7840	7860	7880	7900	7920	7940	7960	7980	8000	8020	8040	8060	8080	8100	8120	8140	8160	8180	8200	8220	8240	826
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2dVH-TTCH native cloned	151	GTGATATGGAGTGGTGGAGGCACGGCCTATACTGCGGGTTTCATATCCAG	200
2dVH-TTCH_N87D_cloned	151	200
2dVH-TTCH_N87D_G53C_cloned	151	200
2dVH-TTCH_N87D_G54C_cloned	151	200
2dVH-TTCH_N87D_G55C_cloned	151	200
2dVH-TTCH expected sequence	151	200
2d12.5 VH native hybridoma	145	194
2dVH-TTCH native cloned	201	ACTGAACATCTACAAGGACAAATCCCAAGCAAGTTTCTTTGAAATGA	250
2dVH-TTCH_N87D_cloned	201	250
2dVH-TTCH_N87D_G53C_cloned	201	250
2dVH-TTCH_N87D_G54C_cloned	201	250
2dVH-TTCH_N87D_G55C_cloned	201	250
2dVH-TTCH expected sequence	201	250
2d12.5 VH native hybridoma	195	244
2dVH-TTCH native cloned	251	ACAGTCTGCAAGCTAATGACACAGCCATGTATTACTGTGCCAGAAGGGGT	300
2dVH-TTCH_N87D_cloned	251	300
2dVH-TTCH_N87D_G53C_cloned	251	300
2dVH-TTCH_N87D_G54C_cloned	251	300
2dVH-TTCH_N87D_G55C_cloned	251	300
2dVH-TTCH expected sequence	251	300
2d12.5 VH native hybridoma	245	294
2dVH-TTCH native cloned	300	ACAGTCTGCAAGCTAATGACACAGCCATGTATTACTGTGCCAGAAGGGGT	350
2dVH-TTCH_N87D_cloned	300	350
2dVH-TTCH_N87D_G53C_cloned	300	350
2dVH-TTCH_N87D_G54C_cloned	300	350
2dVH-TTCH_N87D_G55C_cloned	300	350
2dVH-TTCH expected sequence	300	350
2d12.5 VH native hybridoma	294	344

FIG. 9A (CONT.)

Mouse 2D12.5 VH - Human TetTox CH1 (heavy chain Fab gene)

2dVH-TTCH native cloned	301	AGCTACCCTTACAAC	350
2dVH-TTCH_N87D_cloned	301	CTTACTTCTGGGGCCAAAGGACCA	350
2dVH-TTCH_N87D_G53C_cloned	301	CGGTCTTCCCGCAGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCCT	350
2dVH-TTCH_N87D_G54C_cloned	301	CGGTCTTCCCGCAGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCCT	350
2dVH-TTCH_N87D_G55C_cloned	301	CGGTCTTCCCGCAGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCCT	350
2dVH-TTCH expected sequence	301	CGGTCTTCCCGCAGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCCT	350
2d12.5 VH native hybridoma	295	CGGTCTTCCCGCAGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCCT	344

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2dVH-TTCH native cloned	351	CGGTCTTCCCGCAGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCCT	400
2dVH-TTCH_N87D_cloned	351	CGGTCTTCCCGCAGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCCT	400
2dVH-TTCH_N87D_G53C_cloned	351	CGGTCTTCCCGCAGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCCT	400
2dVH-TTCH_N87D_G54C_cloned	351	CGGTCTTCCCGCAGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCCT	400
2dVH-TTCH_N87D_G55C_cloned	351	CGGTCTTCCCGCAGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCCT	400
2dVH-TTCH expected sequence	351	CGGTCTTCCCGCAGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCCT	400
2d12.5 VH native hybridoma	345	CGGTCTTCCCGCAGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCCT	354

2dVH-TTCH native cloned	401	CCTCCAAGAGACACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAG	450
2dVH-TTCH_N87D_cloned	401	CCTCCAAGAGACACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAG	450
2dVH-TTCH_N87D_G53C_cloned	401	CCTCCAAGAGACACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAG	450
2dVH-TTCH_N87D_G54C_cloned	401	CCTCCAAGAGACACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAG	450
2dVH-TTCH_N87D_G55C_cloned	401	CCTCCAAGAGACACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAG	450
2dVH-TTCH expected sequence	401	CCTCCAAGAGACACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAG	450
2d12.5 VH native hybridoma		CCTCCAAGAGACACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAG	450

FIG. 9B

2dVH-TTCH_native cloned
2dVH-TTCH_N87D cloned

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Mouse 2D12.5 VH - Human TetTox CH1 (heavy chain Fab gene)					
2dVH-TTCH N87D_G53C_cloned	601	660	670	680
2dVH-TTCH N87D_G54C_cloned	601	660	670	680
2dVH-TTCH N87D_G55C_cloned	601	660	670	680
2dVH-TTCH expected sequence	601	660	670	680
2d12.5 VH native hybridoma					
2dVH-TTCH native cloned	651	660	670	680
2dVH-TTCH N87D_cloned	651	660	670	680
2dVH-TTCH N87D_G53C_cloned	651	660	670	680
2dVH-TTCH N87D_G54C_cloned	651	660	670	680
2dVH-TTCH N87D_G55C_cloned	651	660	670	680
2dVH-TTCH expected sequence	651	660	670	680
2d12.5 VH native hybridoma					

FIG. 9C

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Strategy for Assembly of Chimeric 2D12.5
 Heavy Chain
 Step 1

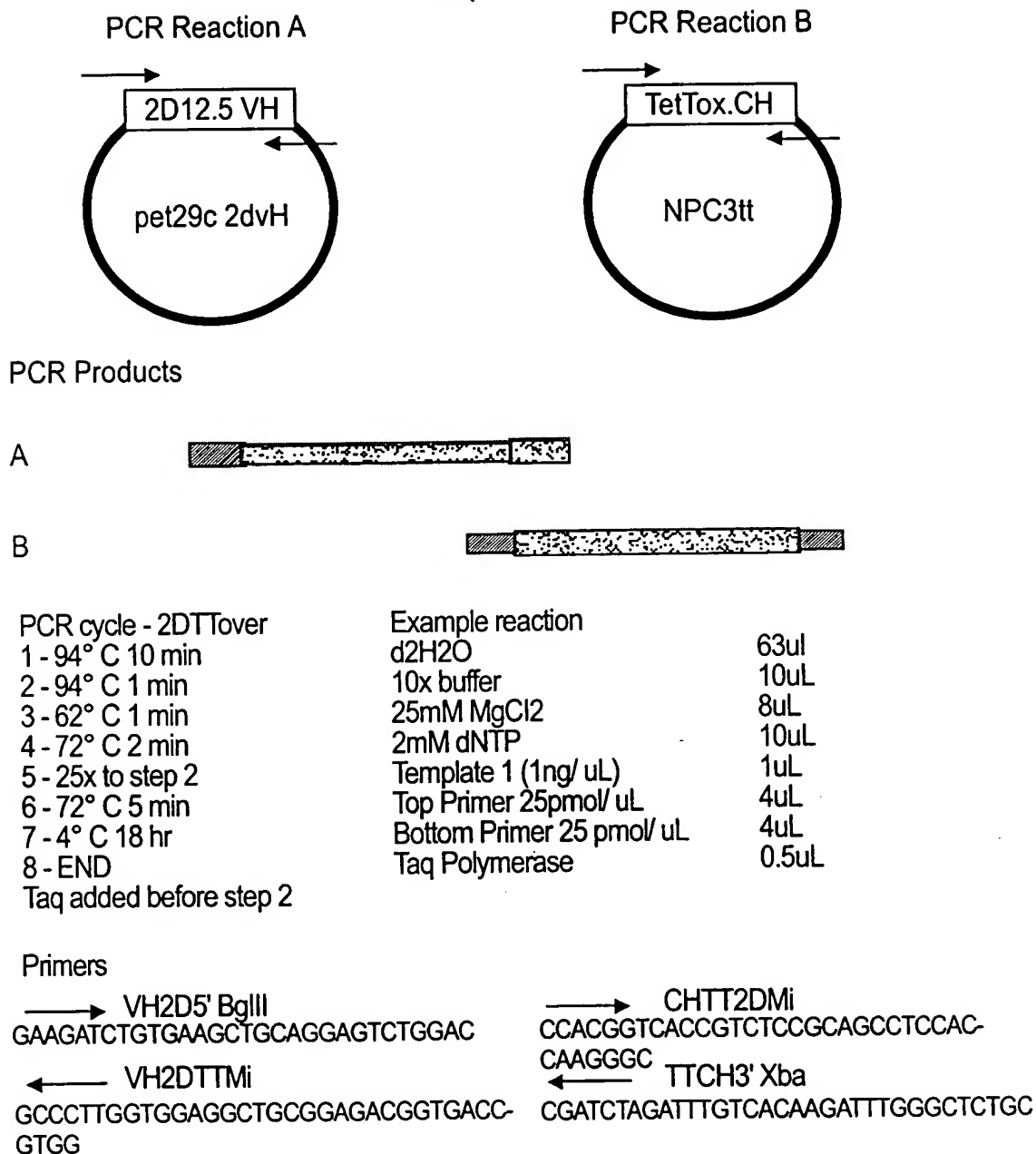
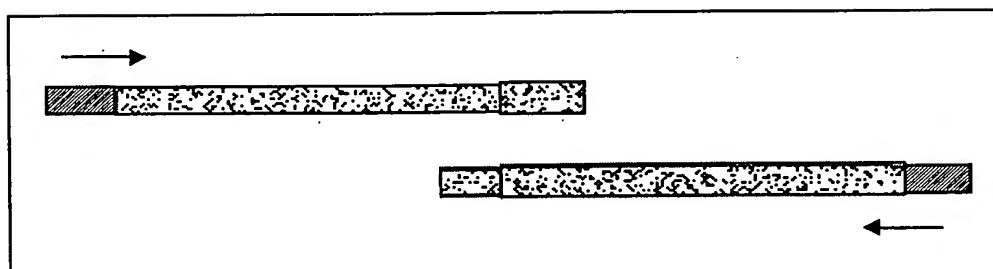


FIG. 10A

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Strategy for Assembly of Chimeric 2D12.5
 Heavy Chain
 Step 2

PCR Reaction



PCR cycle - 2DTTVent

- 1 - 95°C 10 min
 - 2 - 94°C 1 min
 - 3 - 60°C 1 min
 - 4 - 75°C 2 min
 - 5 - 4x to step 2
 - 6 - 94°C 1 min
 - 7 - 63°C 1 min
 - 8 - 75°C 2 min
 - 9 - 25x to step 6
 - 10 - 72°C 5 min
 - 11 - 4°C 18 hr
 - 12 - END
- Vent added before step 2
 Primers added before step 6

Primers

→ VH2D5' BglII
 GAAGATCTGTGAAGCTGCAGGAGTCTGGACC

← TTCH3' Xba
 CGATCTAGATTTGTCACAAGATTGGGCTCTGC

Example reaction

d2H2O	70uL
10x buffer	10uL
100mM MgSO4	0uL
2mM dNTP	10uL
Template 1(1ng/ uL)	1uL
Template 2(1ng/ uL)	1uL
Top Primer 25pmol/ uL	4uL
Bottom Primer 25 pmol/ uL	4uL
Vent Polymerase	0.5uL

PCR Assembly Product



FIG. 10B

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Strategy for Assembly of Chimeric 2D12.5
Heavy Chain
Step 3

Desired PCR Assembly Product



Restriction Digest PCR Product with Bgl II & Xba I



Ligate Restriction Digested PCR Product into pMTBipV5His
(S2 Cell Expression Vector, Propagated in XL-1 Blue E. Coli)

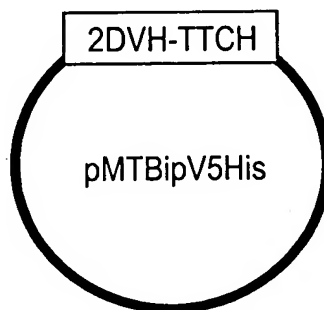
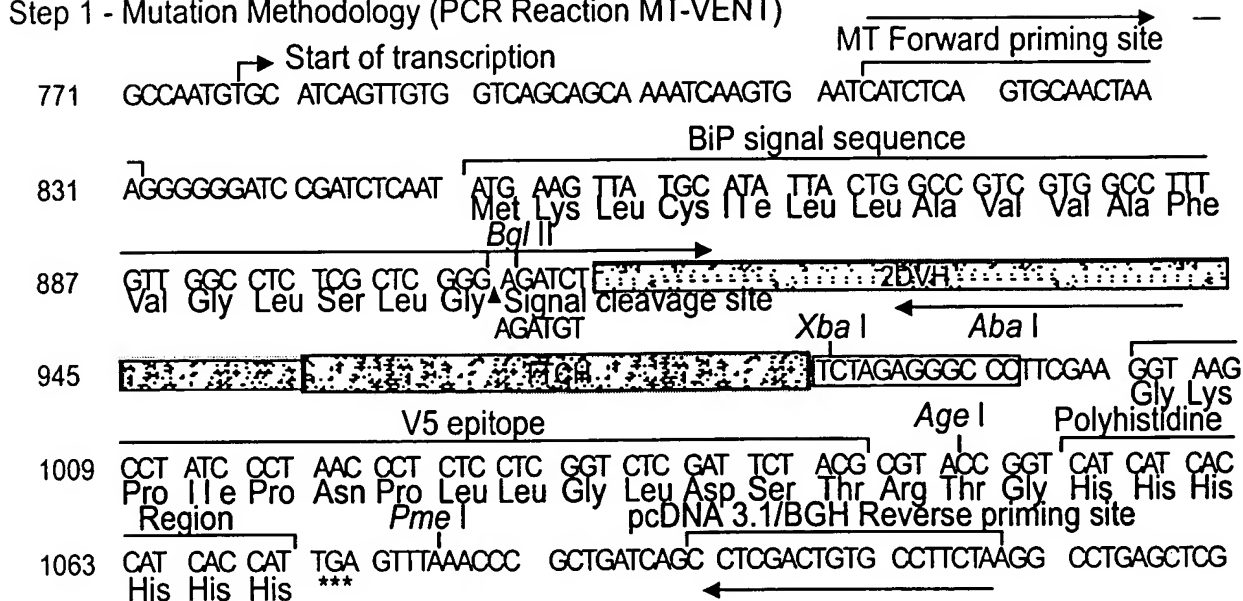


FIG. 10C

Step 1 - Mutation Methodology (PCR Reaction MT-VENT)



Rxn A

Mutation

CATCTCAGTGCAACTAAA MTforward

CATGGCTGTGTCATCAGCTTGCAGACTGTTC 2dvhN87D_pMTBip

CGTGCCTCCACAACCTCCATATCAC G53C noncoding 2dG53c_pMT

CCGTGCCACAACCACTCCATATC G54C noncoding 2dG54c_pMT

CCGTGCATCCACCACTCCATATC G55C noncoding 2dG55c_pMT

Rxn B

Killed Bgl II

GCTCGGGAGATGTGTGAAGCTG 2dvhKBglIII_pMTBip

TAGAAGGCACAGTCGAGG BGHreverse

FIG. 1

FIG. 10D

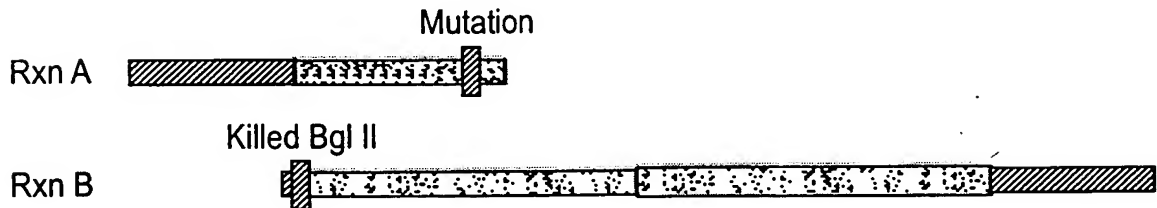
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Step 2 - Mutation Methodology (PCR Reaction VHMUTTAQ)

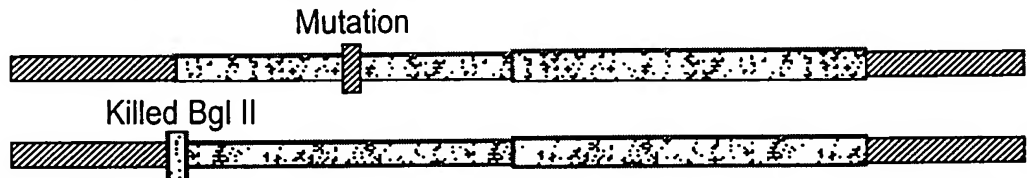
2nd PCR Reaction (Mix Products of reaction A and B)

1) Extend

2) Amplify with outer primers (MTforward and BGHreverse)



2nd PCR Reaction Products (Mixture - 2 Products of equal size)



Restriction Digest PCR Product Mixture with BglII and Xba1

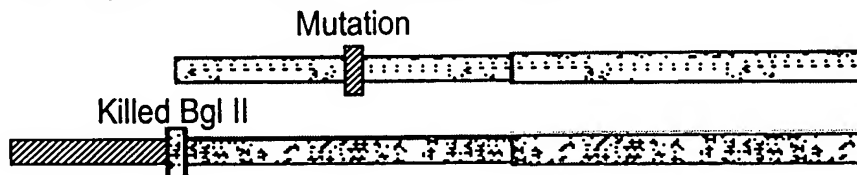


FIG. 10E

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Step 1 - PCR Reaction MT-VENT

PCR cycle - MT-VENT

1 - 95° C 10 min
2 - 94° C 1 min
3 - 50° C 1 min
4 - 75° C 2 min
5 - 24x to step 2
6 - 75° C 5 min
7 - 4° C 18 hr
8 - END
VENT added before step 2
Primers added before step 1

Example reaction

d2H2O	70ul
10x buffer	10uL
100mM MgSO4	0uL
2mM dNTP	10uL
Template (1ng/uL)	1uL
Top Primer 25pmol/uL	4uL
Bottom Primer 25pmol/uL	4uL
Vent Polymerase	0.5uL

Step 2 - PCR Reaction VHMUTTAQ

PCR cycle - VHMUTTAQ

1 - 95° C 10 min
2 - 94° C 1 min
3 - 68° C 1 min
4 - 72° C 2 min
5 - 4x to step 2
6 - 94° C 1 min
7 - 50° C 2 min
8 - 72° C 2 min
9 - 24x to step 6
10 - 72° C 5 min
11 - 4° C 18 hr
12 - END
Taq added before step 2
Primers added before step 6

Example reaction

d2H2O	61ul
10x buffer	10uL
25mM MgCl2	8uL
2mM dNTP	10uL
Template 1 (1ng/uL)	1uL
Template 2 (1ng/uL)	1uL
Top Primer 25pmol/uL	4uL
Bottom Primer 25pmol/uL	4uL
Taq Polymerase	0.5uL

FIG. 10F

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Strategy for Assembly of Chimeric 2D12.5
 Light Chain
 Step 1

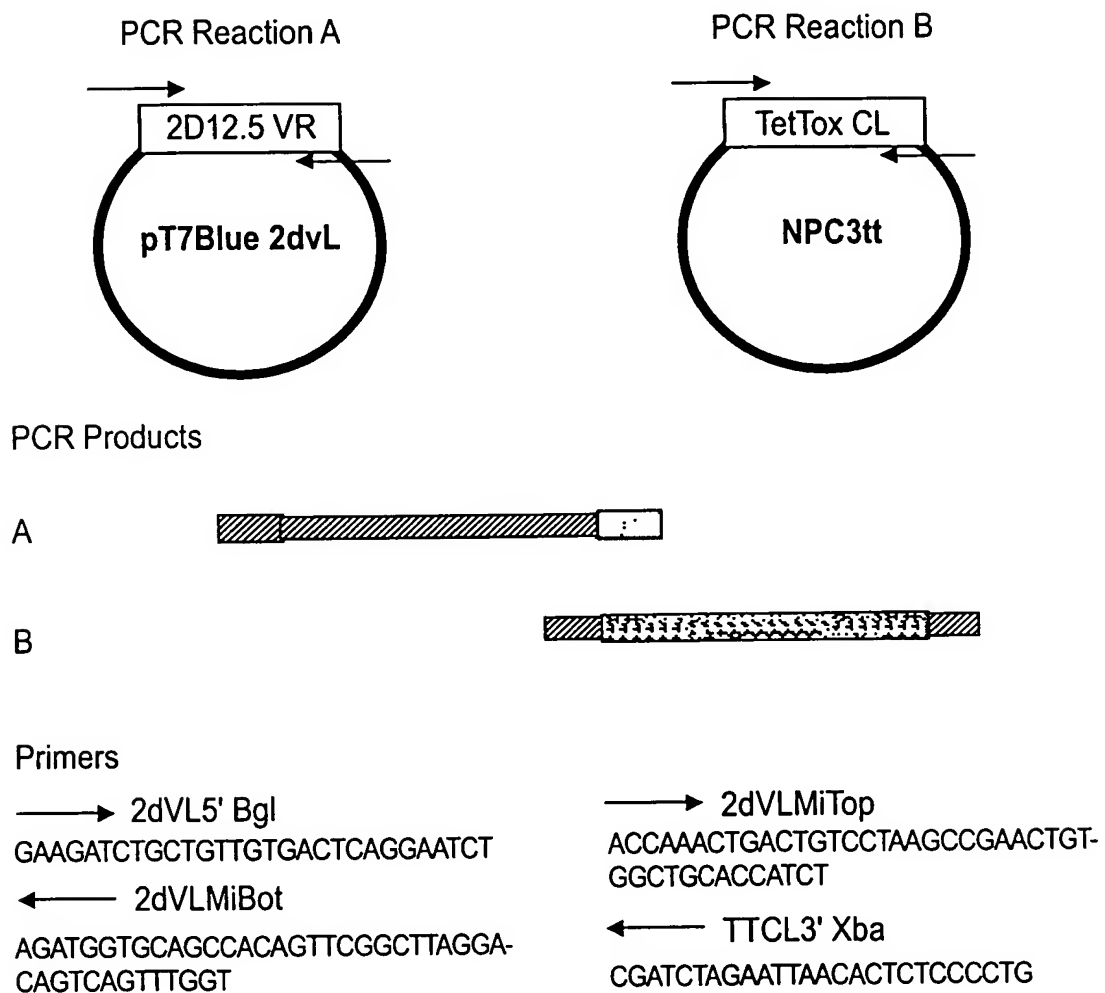
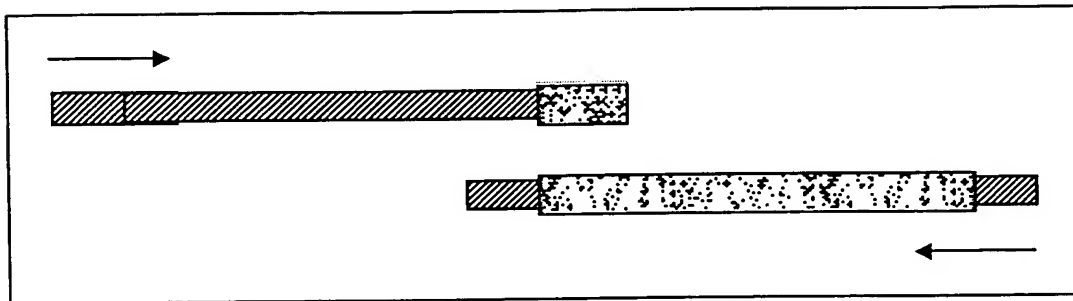


FIG. 11A

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Strategy for Assembly of Chimeric 2D12.5
Light Chain
Step 2

PCR Reaction



Primers

→ 2dVL5' Bgl
GAAGATCTGCTGTTGTGACTCAGGAATCT

← TTCL3' Xba
CGATCTAGAATTAACACTCTCCCCTG

PCR Assembly Product



Problem: There is an unwanted G48S mutation in the variable domain.

Solution: Repair with an S48G primer using the method of Ito. Also institute a mutation at N53C and repair point mutation with a single primer named S48G_N53C.

FIG. 11B

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Strategy for Assembly of Chimeric 2D12.5
Light Chain
Step 3

Desired PCR Assembly Product



Restriction Digest PCR Product with Bgl II & Xba I



Ligate Restriction Digested PCR Product into pMTBipV5His
(S2 Cell Expression Vector, Propagated in XL-1 Blue E. Coli)

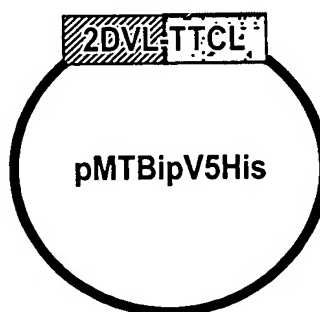


FIG. 11C

MT Forward priming site

1st Set of PCR Reactions Product A and B)

Killed Bgl II

Rxn B

GCTCGGGAGATGTGCTGTTGTG

2dvLKgIII_pMTBip

TAGAAGGCACAGTCGAGG

BGHreverse

FIG. 11D

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Step 2 - Mutation Methodology (PCR Reaction VHMUTTAQ)

2nd PCR Reaction Mix Products of reaction B)

1) Extend

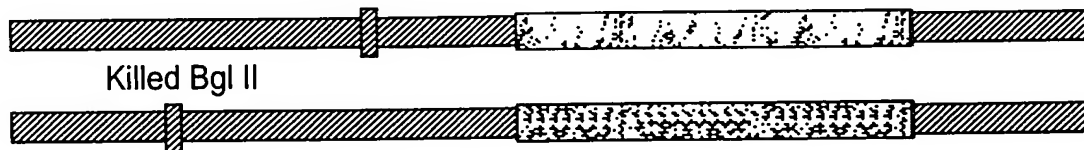
2) Amplify with outer primers (MTforward and BGHreverse)

Mutation



2nd PCR Reaction Products (Mixture - 2 Products of equal size)

Mutation



Restriction Digest PCR Product Mixture with BglII and Xba1

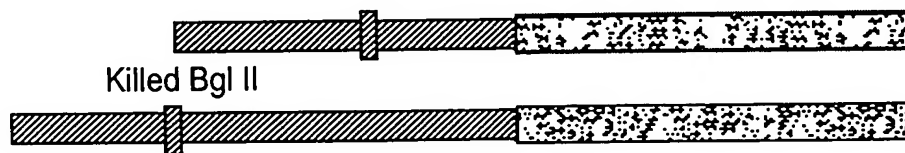


FIG. 11E

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Strategy for Assembly of Chimeric 2D12.5 Light Chain

Step 4

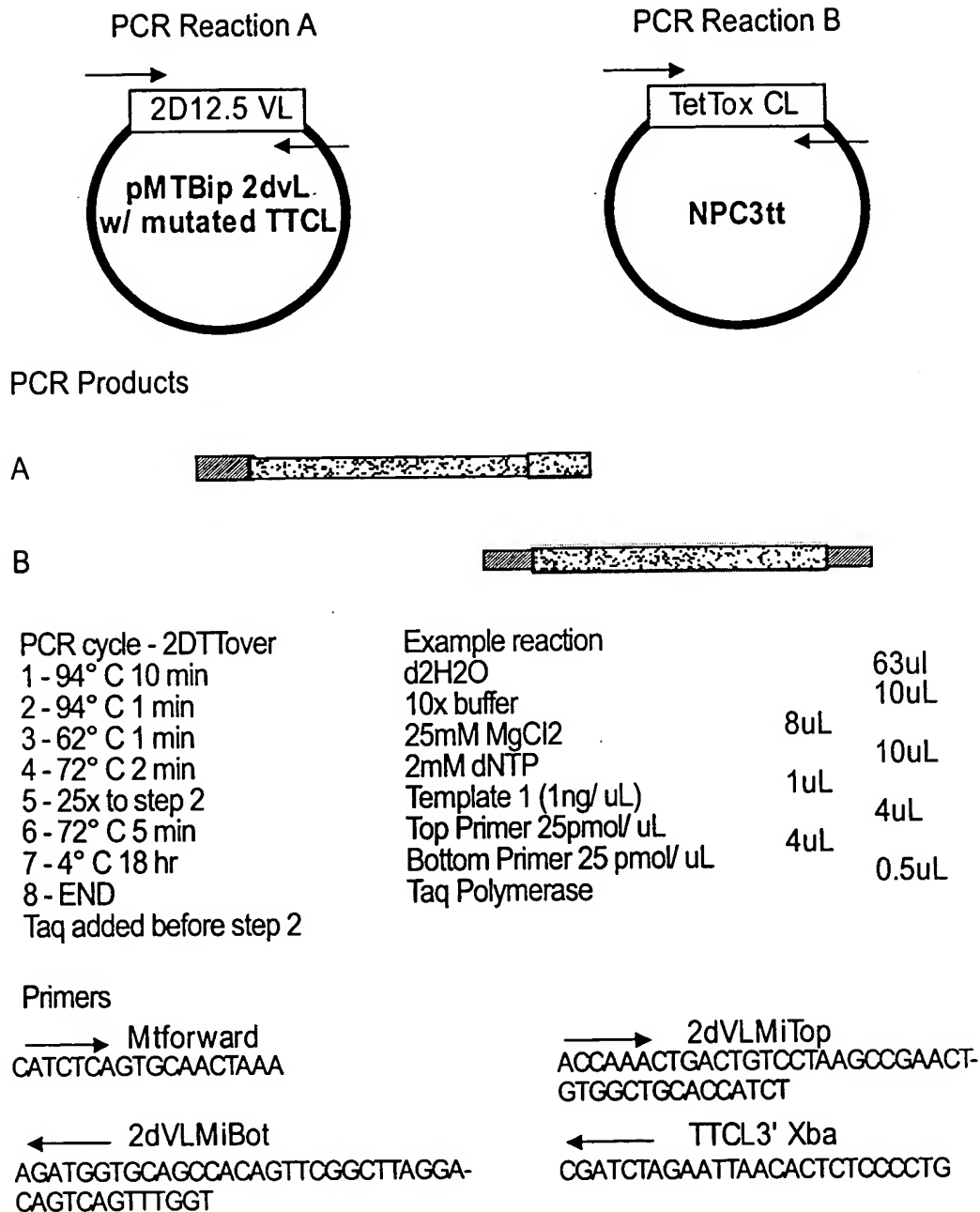


FIG. 11F

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Assembled Vectors for Transfection in S2 Cells

Each of the following has been cotransfected
with the native light chain:

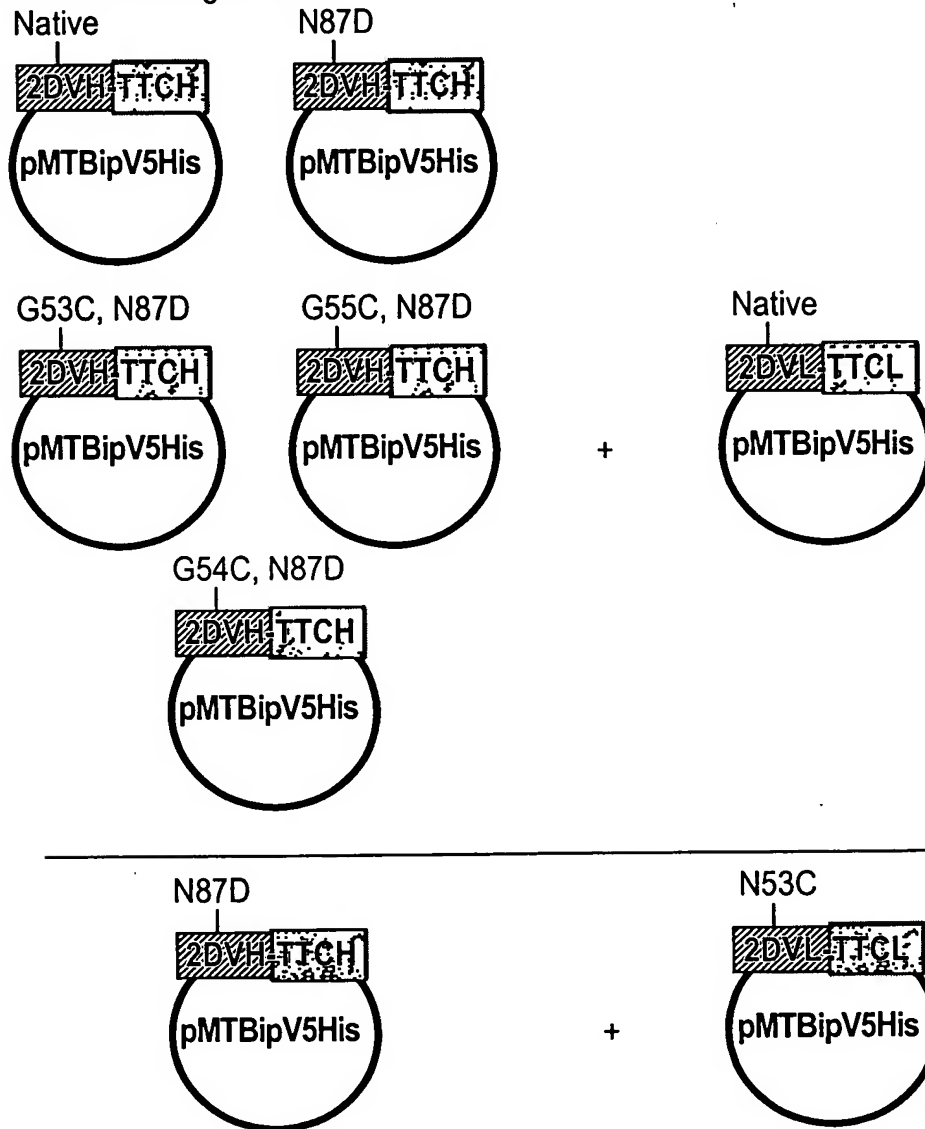


FIG. 11G

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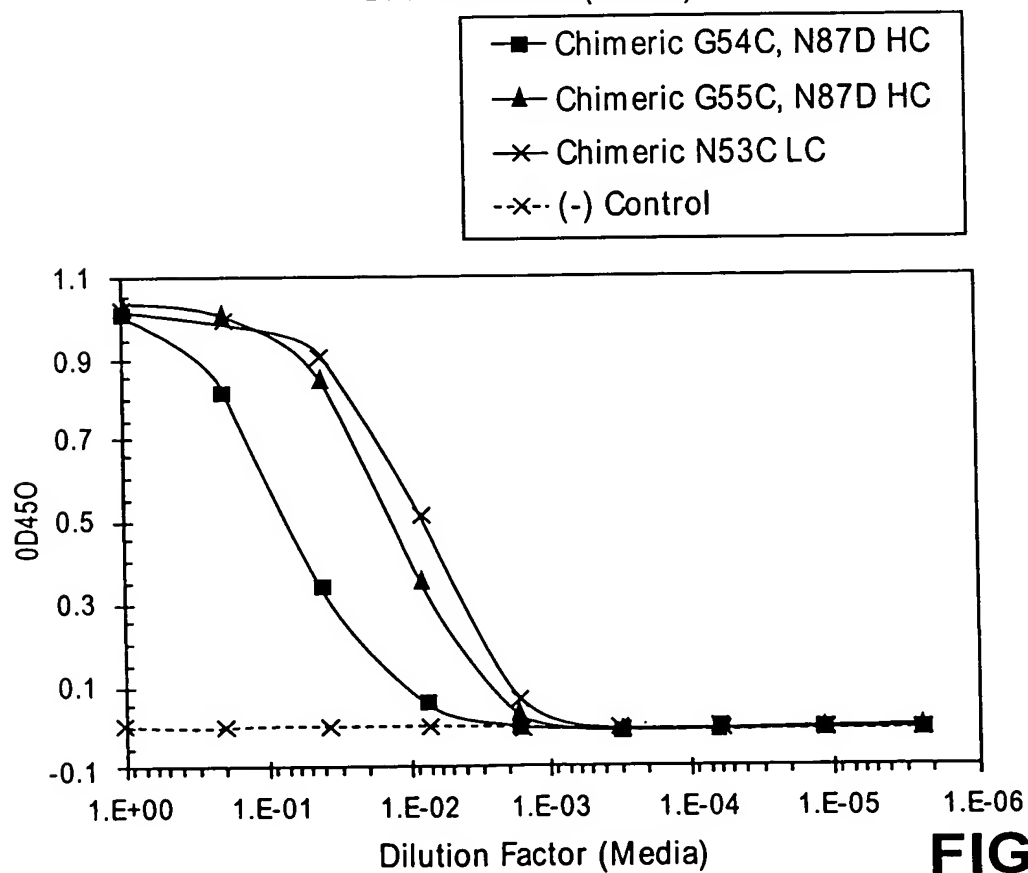
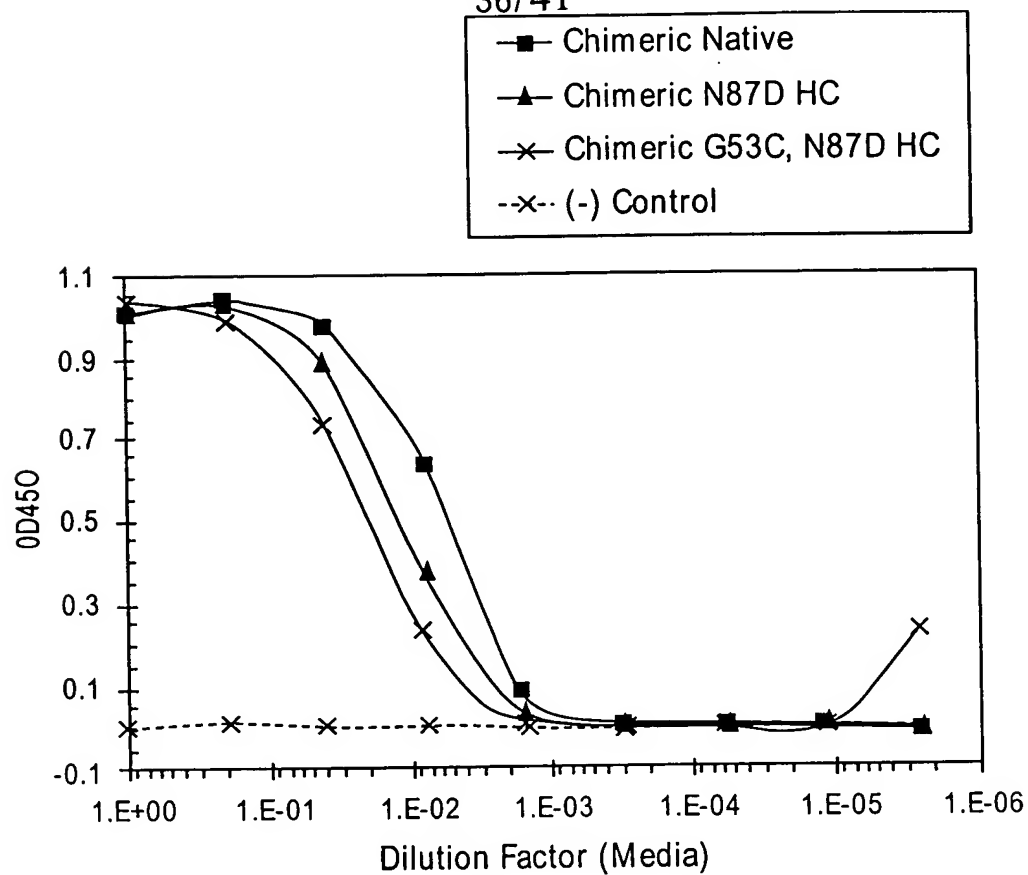


FIG. 12

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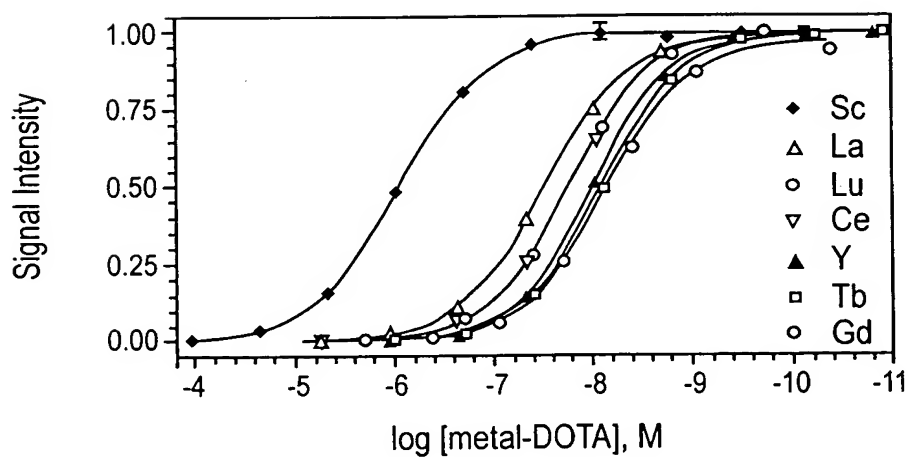


FIG. 13

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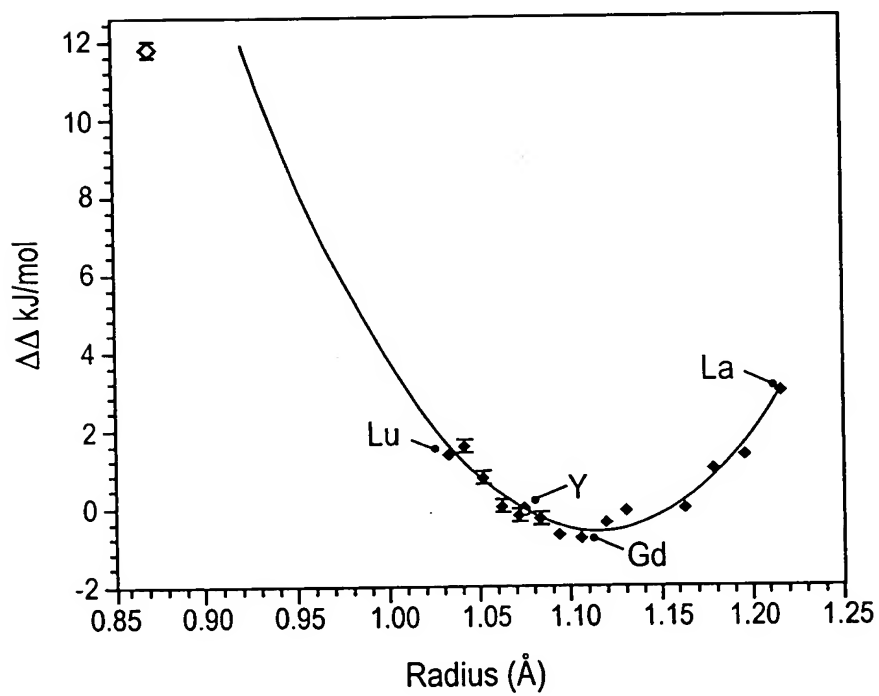


FIG. 14

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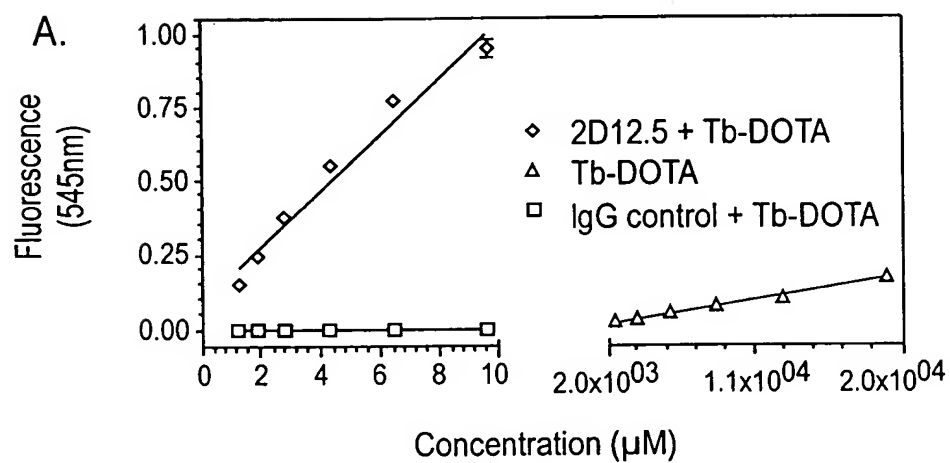


FIG. 15

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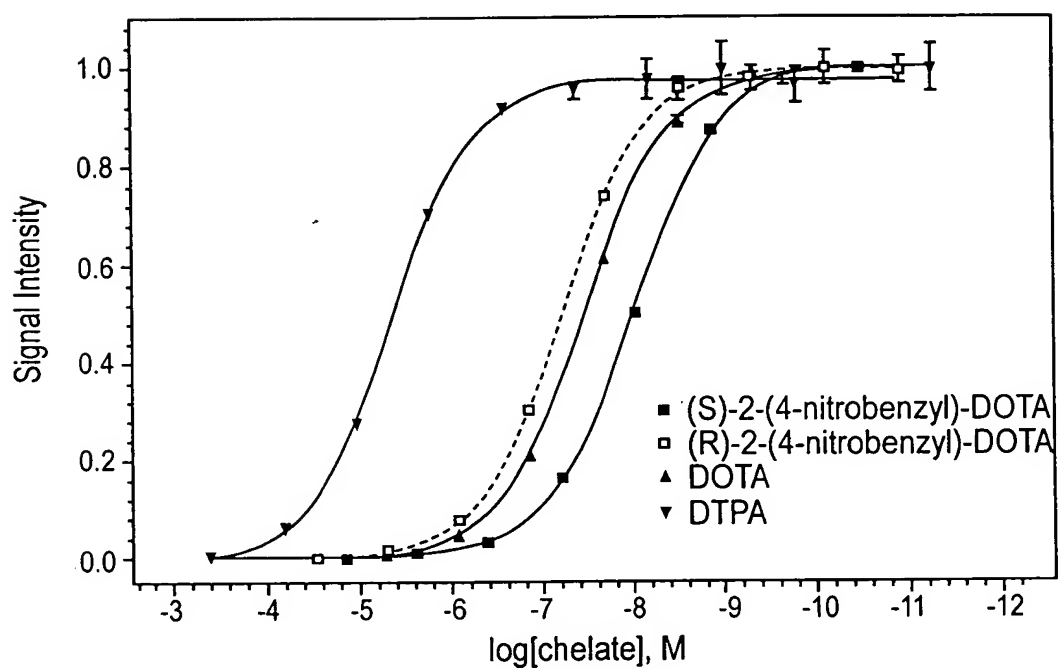


FIG. 16

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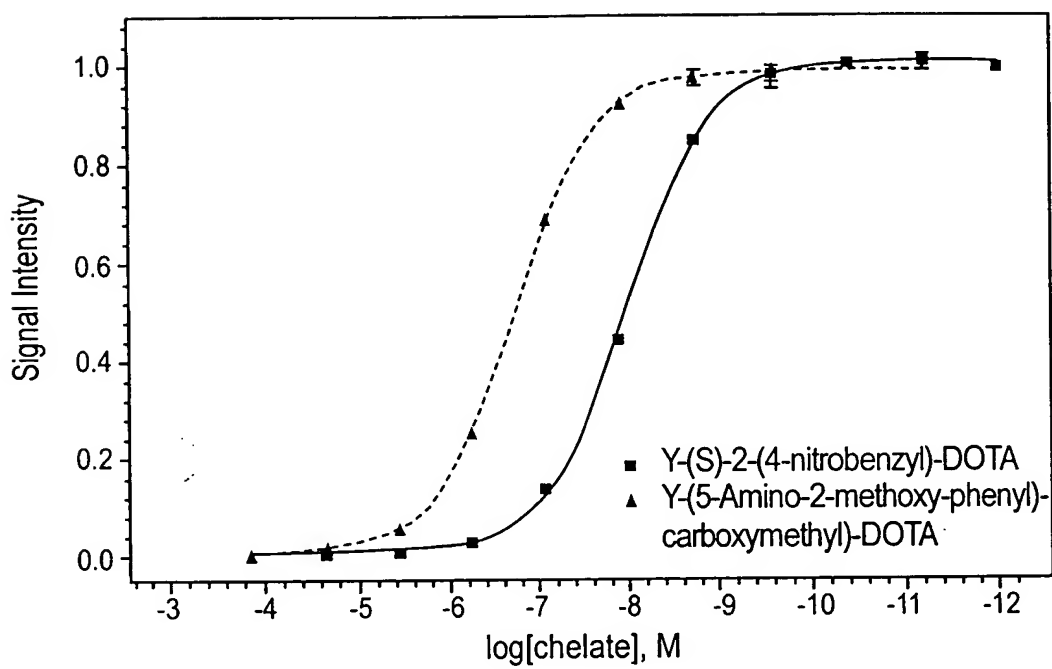


FIG. 17